(3) *Resolution factor.* Calculate the resolution factor (R) as follows:

$$R = \frac{2(t_{Rj} - t_{Ri})}{w_i + w_j}$$

where:
t_{Rj}=Retention time for a solute eluting after i (t_{Rj} is larger than t_{Ri});
t_{Ri}=Retention time for any solute;
w_i=Width of peak at baseline for any solute; and
w_j=Width of peak at baseline for any solute eluting after i.

(4) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation (S_R

$$S_R = \frac{100}{\overline{X}} \left(\frac{\sum_{i=1}^{n} \left(X_i - \overline{X} \right)^2}{N-1} \right)^{1/2}$$

where:
$\overline{X}$ is the mean of N individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using the sample solution in lieu of the working standard solution.

[50 FR 7764, Feb. 26, 1985; 50 FR 10220, Mar. 14, 1985; 50 FR 18243, Apr. 30, 1985]

§ 436.354 High-performance liquid chromatographic assay for ceftriaxone.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 3 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested.

(b) *Procedure.* Perform the assay at the temperature specified in the monograph for the drug being tested with a flow rate not to exceed 2.0 milliliters per minute, Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale. Use the apparatus described in paragraph (a) of this section; and also, use the system suitability requirements, reagents, working standard, test and sample solutions, and calculations as directed in the individual monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of 20 microliters each of the test solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements as described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Using the apparatus and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Capacity factor.* Calculate the capacity factor (k) as follows:

$$k = \frac{t_R - t_M}{t_M}$$

where:
t_R=Retention time of solute; and
t_M=Retention time of solvent or unretained substance.

(2) *Resolution.* Calculate the resolution (R) as follows:

$$R = \frac{2(t_{Rj} - t_{Ri})}{w_i + w_j}$$

where:
t_{Rj}=Retention time for a solute eluting after i (t_{Rj} is larger than t_{Ri});
t_{Ri}=Retention time for any solute;
w_i=Width of peak at baseline for any solute; and
w_j=Width of peak at baseline for any solute eluting after i.

(3) *Asymmetry factor.* Calculate the asymmetry factor (A_s

$$A_s = \frac{a+b}{2a}$$

where:
a=Horizontal distance from point of ascent to point of maximum peak height; and

b=Horizontal distance from point of maximum peak height to point of descent.

(4) *Efficiency of the column.* Calculate the efficiency of the column (reduced plate height) (h_r

(i)

$$n = 5.545\left(\frac{t_R}{W_h}\right)^2;$$

(ii)

$$h = \frac{L}{n}; \text{ and}$$

(iii)

$$h_r = \frac{h}{d_p}.$$

where:

n=Efficiency, as number of theoretical plates for column;

t_R=Retention time of solute;

w_h=Peak width at half-height;

h=Efficiency, as height equivalent to one theoretical plate;

L=Length of column; and

d_p=Average diameter of particle in column.

(5) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation (S_R

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:

$\overline{X}$ is the mean of N individual measurements of X_i.

The complete operating system is acceptable for assay if it meets the system suitability requirements of the monograph for the drug being tested. If the complete operating system is acceptable, proceed as described in paragraph (b) of this section using the sample solution in lieu of the test solution. Calculate the drug content as described in the individual monograph for the drug being tested.

[50 FR 9999, Mar. 13, 1985]

§ 436.355 High-performance liquid chromatographic assay for ticarcillin-clavulanic acid.

(a) *Equipment.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being testing;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 10 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, and calculations specified in the monograph for the drug being tested with a flow rate not to exceed 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the equipment described in paragraph (a) of this section; and the reagents and working standard and sample solutions described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. The clavulanic acid peak is sharp and the chromatograms of standard and sample solutions show baseline separations between it and any neighboring peaks. The retention times for clavulanic acid and ticarcillin are approximately 3 minutes and 14 minutes, respectively. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.